IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Not yet assigned
Group	:	Not yet assigned
Applicants	:	Christian Plank et al.
Application No.	:	Not yet assigned
Filed	:	Concurrently herewith
For	:	COMBINATIONS FOR INTRODUCING NUCLEIC ACIDS INTO CELLS

New York, New York
December 17, 2001

BOX PATENT APPLICATION
Hon. Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202

PRELIMINARY AMENDMENT

Sir:

Prior to the issuance of the first Office Action in the above-identified application, kindly amend the application as follows:

IN THE SPECIFICATION

On page 1, on the first line of the application after the title, insert the following paragraph:

This application claims benefit under 35 U.S.C. § 120 from International Application No. PCT/EP00/05778, filed June 21, 2000.

On page 22, replace the paragraph beginning "**Fig. 14:**" with the following paragraph:*

Fig. 14: Release of radioactive-labeled DNA from vector-loaded collagen sponges. The sponges were prepared as described in Example 18. In the case of naked DNA, approximately 50 % of the applied dose bind actively, whereas the other half is immediately released. The subsequent release kinetics follows an approximately linear course. If gene vectors are loaded on sponges, a fraction of 90 % is bound tightly and is released over an extended time period with an exponential release profile. Cationically derivatized sponges ("PEI-SPDP" and "Peptide-SPDP") bind naked DNA efficiently and display release kinetics similar to vector-loaded sponges.

Replace the paragraph beginning at the bottom of page 22 and continuing to page 23 with the following paragraph:

Fig. 15: Gene transfer into NIH3T3 mouse fibroblasts by vector-loaded collagen sponges. The sponges were prepared as described in Example 16 (naked DNA, PEI-DNA, DOTAP-cholesterol-DNA prepared according to the variant procedure) and used for gene delivery as described in Example 19. In the case of DOTAP-cholesterol sponges, the preparations were either added to an adherent layer of cells (left), or freshly trypsinized cells were loaded on the

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* Applicants enclose a "Version Showing Changes Made" including the amendments to the specification and to the claims.

sponge (right). The subsequent experimental course was identical for all setups. The reporter gene expression was assayed over various time spans and persists over extended periods particularly in cells growing on/in the sponges.

Replace the paragraph beginning at the bottom of page 45 and continuing to page 46 with the following paragraph:

a) Control (PEI-DNA, N/P = 8):

150 µg DNA (pCLuc) in 337.5 µl 20 mM HEPES pH 7.4 were pipetted to 156.4 µg of PEI (25 kD, Aldrich) in the same volume of HEPES buffer. After 15 min, 75 µl 50 % glucose were added. Of this solution, 100 µl each were injected into the tail vein of mice (corresponding to 20 µg DNA per animal).

Replace the second paragraph on page 46 with the following paragraph:

b) Control (DOTAP/cholesterol-DNA; charge ratio +/- = 5):

DOTAP-cholesterol liposomes were prepared according to a standard protocol (Barron et al., 1998). In this case, liposomes with a molar ratio of DOTAP to cholesterol of 1:1 and a final concentration of 5 mM DOTAP in 5 % glucose were prepared. 130 µg DNA in 191.1 µl 20 mM HEPES pH 7.4 were added to 393.5 µl liposome suspension. After 15 min, 65 µl 50 % glucose were added. Of this solution, 100 µl each were injected into the tail vein of mice (corresponding to a dose of 20 µg DNA per animal).

Replace the paragraph beginning at the bottom of page 59 and continuing to page 60 with the following paragraph:

The table shows gene transfer in vivo upon subcutaneous implantation of sponge preparations. The sponges were prepared as described in Examples 15 and 16, respectively, and were implanted subcutaneously in Wistar rats as described in Example 17. The gene expression first of all was determined after 3 days. Only collagen sponges loaded with PEI-DNA complexes coated with a copolymer of the invention give rise to detectable reporter gene expression under this experimental setup (numbers are fg luciferase/mg protein).

Replace the second paragraph on page 60 with the following paragraph:

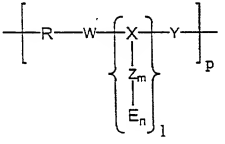
EXAMPLE 18: Release of radioactive-labeled DNA from various collagen sponge - vector preparations

IN THE CLAIMS

On page 71, line 1 after the word "Claims," insert the following paragraph: "We claim:".

Replace claims 1-9 and 12-15, with substitute claims 1-9 and 12-15 as follows:

1. (Amended) A combination of a carrier and a complex comprising a nucleic acid molecule and a charged copolymer of the general formula I

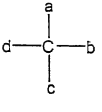


wherein R is an amphiphilic polymer or a homo- or hetero-bifunctional derivative thereof,

and wherein X

i) is an amino acid or an amino acid derivative, a peptide or a peptide derivative or a spermine or a spermidine derivative; or

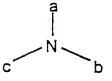
ii) wherein X is



wherein

a is H or, optionally halogen- or dialkylamino-substituted, C_1-C_6 alkyl; and wherein b, c and d are the same or different, optionally halogen- or dialkylamino-substituted, C_1-C_6 alkylene; or

iii) wherein X is



wherein

a is H or, optionally halogen or dialkylamino substituted, C_1-C_6 alkyl,

and wherein

b and c are the same or different, optionally halogen- or dialkylamino-substituted, C_1-C_6 alkylene; or

iv) wherein X

is a substituted aromatic compound with three functional groupings $W_1Y_1Z_1$, wherein W, Y and Z have the meanings mentioned below;

wherein

W, Y or Z have the same or different groups CO, NH, O or S or a linker grouping capable of reacting with SH, OH, NH or NH_2;

and wherein the effector molecule E

is a cationic or anionic peptide or peptide derivative or a spermine or spermidine derivative or a glycosaminoglycane or a non-peptidic oligo/polycation or -anion; wherein

m and n are independently of each other 0, 1 or 2; wherein

p preferably is 3 to 20; and wherein

l is 1 to 5, preferably 1.

2. (Amended) The combination according to claim 1, wherein the amphiphilic polymer is a polyalkylene oxide.

3. (Amended) The combination according to claim 2, wherein the amphiphilic polymer is a polyalkylene glycol.

4. (Amended) The combination according to any one of claims 1 to 3, wherein X or E is a charged peptide or peptide derivative.

5. (Amended) The combination according to any one of claims 1 to 3, wherein a ligand for a higher eukaryotic cell is coupled to the copolymer.

6. (Amended) The combination according to any one of claims 1 to 3, wherein the nucleic acid molecule is condensed with an organic polycation or cationic lipid

molecule and the complex formed thereby has a charged copolymer of the general formula I bound to its surface via ionic interaction.

7. (Amended) The combination according to any one of claims 1 to 3, containing a therapeutically effective nucleic acid molecule.

8. (Amended) The combination according to any one of claims 1 to 3, wherein the carrier consists of a biologically non-resorbable material.

9. (Amended) The combination according to any one of claims 1 to 3, wherein the carrier consists of a biologically resorbable material.

12. (Amended) The combination according to any one of claims 1 to 3, wherein the carrier is a carrier which is obtainable by cross-linkage of a copolymer as defined in claim 1.

13. (Amended) A method of transferring a nucleic acid molecule into a cell comprising using a combination according to any one of claims 1 to 3.

14. (Amended) A pharmaceutical composition comprising a combination according to any one of claims 1 to 3.

15. (Amended) A kit comprising a carrier and a copolymer or a complex as defined in claim 1.

REMARKS

Applicants have amended the specification to set forth a priority claim under 35 U.S.C. § 120 to an earlier-filed copending international application designating the United States.

Applicants have further amended the specification to correct typographical errors. The application as filed contained two examples designated as Example 17 and no Example 18. Therefore, applicants have renumbered the second such example as Example 18 and, accordingly, applicants have amended references to the examples in the figure legends and in the text of the first example numbered Example 17.

Applicants also have amended two typographical errors in Example 13. First, applicants have amended Example 13(a) to recite 156.4 μg PEI in place of 156.4 μl PEI. The specification refers to quantities of PEI by both mass and volume, but it clearly indicates when solutions of PEI are used. See, for example, page 34, line 13; page 37, line 10; page 41, line 28; page 42, line 6; and page 46, line 12; page 47, line 23. The text of Example 13(a) clearly indicates that the amount of PEI used is present in a specified volume of buffer and therefore that a solution of PEI is being made rather than used. Furthermore, the example states that the PEI in Example 13(a) is present in the same volume of HEPES buffer as the solution containing DNA (i.e. each is present in 337.5 μl for a total volume of 675 μl). It would be clear to one of skill in the art that to achieve a dosage of 20 μg DNA/100 μl injection, which is the dosage consistently used in this example (see page 46, lines 2, 10, 20, and 25-26), the final volume of the solution must be approximately 750 μl. Therefore, one having ordinary skill in the art would recognize that the use of the unit "μl" was a mistake and that "μg" was intended because the addition of 156.4 μl PEI would result in a final

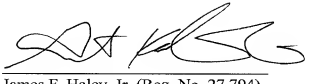
volume of 906.4 µl (337.5 µl + 337.5 µl + 156.4 µl + 75 µl glucose solution) and thus a dosage of only 16.5 µg DNA/100 µl injection.

Second, applicants have amended Example 13(b) to recite that the DNA is present in 191.1 µl HEPES buffer rather than 91.1 µl. Again, it would be clear to one of skill in the art that to achieve a dosage of 20 µg DNA/100 µl injection the volume of the solution used for inoculation must be approximately 650 µl because it contains 130 µg DNA. Therefore, one of skill in the art would recognize that the volume of the DNA should be 191.1 µl rather than 91.1 µl because the volumes must add up to approximately 650 µl (393.5 µl liposome + 65 µl glucose solution + 191.1 µl DNA = 649.6 µl final volume).

Applicants have amended claims 1-9 and 12-15 to improve their form.

None of these amendments add new matter. Their entry is requested.

Respectfully submitted,



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VERSION SHOWING CHANGES MADE

In the Specification

Paragraph on page 22:

Fig. 14: Release of radioactive-labeled DNA from vector-loaded collagen sponges. The sponges were prepared as described in Example [17] 18. In the case of naked DNA, approximately 50 % of the applied dose bind actively, whereas the other half is immediately released. The subsequent release kinetics follows an approximately linear course. If gene vectors are loaded on sponges, a fraction of 90 % is bound tightly and is released over an extended time period with an exponential release profile. Cationically derivatized sponges ("PEI-SPDP" and "Peptide-SPDP") bind naked DNA efficiently and display release kinetics similar to vector-loaded sponges.

Paragraph spanning pages 22-23:

Fig. 15: Gene transfer into NIH3T3 mouse fibroblasts by vector-loaded collagen sponges. The sponges were prepared as described in Example 16 (naked DNA, PEI-DNA, DOTAP-cholesterol-DNA prepared according to the variant procedure) and used for gene delivery as described in Example [18] 19. In the case of DOTAP-cholesterol sponges, the preparations were either added to an adherent layer of cells (left), or freshly trypsinized cells were loaded on the sponge

(right). The subsequent experimental course was identical for all setups. The reporter gene expression was assayed over various time spans and persists over extended periods particularly in cells growing on/in the sponges.

Paragraph spanning pages 45-46:

a) Control (PEI-DNA, N/P = 8):

150 µg DNA (pCLuc) in 337.5 µl 20 mM HEPES pH 7.4 were pipetted to 156.4 [µl] µg of PEI (25 kD, Aldrich) in the same volume of HEPES buffer. After 15 min, 75 µl 50 % glucose were added. Of this solution, 100 µl each were injected into the tail vein of mice (corresponding to 20 µg DNA per animal).

Second paragraph on page 46:

b) Control (DOTAP/cholesterol-DNA; charge ratio +/- = 5):

DOTAP-cholesterol liposomes were prepared according to a standard protocol (Barron et al., 1998). In this case, liposomes with a molar ratio of DOTAP to cholesterol of 1:1 and a final concentration of 5 mM DOTAP in 5 % glucose were prepared. 130 µg DNA in [91.1] 191.1 µl 20 mM HEPES pH 7.4 were added to 393.5 µl liposome suspension. After 15 min, 65 µl 50 % glucose were added. Of this solution, 100 µl each were injected into the tail vein of mice (corresponding to a dose of 20 µg DNA per animal).

Paragraph spanning pages 59-60:

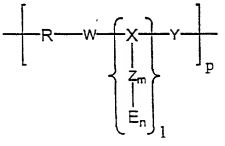
The table shows gene transfer in vivo upon subcutaneous implantation of sponge preparations. The sponges were prepared as described in Examples 15 and 16, respectively, and were implanted subcutaneously in Wistar rats as [discribed] described in Example [16] 17. The gene expression first of all was determined after 3 days. Only collagen sponges loaded with PEI-DNA complexes coated with a copolymer of the invention give rise to detectable reporter gene expression under this experimental setup (numbers are fg luciferase/mg protein).

Second paragraph on page 60:

EXAMPLE [17] 18: Release of radioactive-labeled DNA from various collagen sponge - vector preparations

In the Claims

1. (Amended) A combination of a carrier and a complex [containing one or more] comprising a nucleic acid [molecules] molecule and [one or more] a charged [copolymers] copolymer of the general formula I

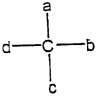


wherein R is an amphiphilic polymer or a homo- or hetero-bifunctional derivative thereof,

and wherein X

i) is an amino acid or an amino acid derivative, a peptide or a peptide derivative or a spermine or a spermidine derivative; or

ii) wherein X is



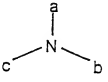
wherein

a is H or, optionally halogen- or dialkylamino-substituted, C_1-C_6 alkyl; and

wherein

b, c and d are the same or different, optionally halogen- or dialkylamino-substituted, C_1-C_6 alkylene; or

iii) wherein X is



wherein

a is H or, optionally halogen or dialkylamino substituted, C_1-C_6 alkyl,

and wherein

b and c are the same or different, optionally halogen- or dialkylamino-substituted, C_1-C_6 alkylene; or

iv) wherein X

is a substituted aromatic compound with three functional groupings $W_1Y_1Z_1$, wherein W, Y and Z have the meanings mentioned below;

wherein

W, Y or Z have the same or different groups CO, NH, O or S or a linker grouping capable of reacting with SH, OH, NH or NH_2;

and wherein the effector molecule E
is a cationic or anionic peptide or peptide derivative or a spermine or spermidine derivative or a glycosaminoglycane or a non-peptidic oligo/polycation or -anion; wherein
m and n are independently of each other 0, 1 or 2; wherein

p preferably is 3 to 20; and wherein

l is 1 to 5, preferably 1.

2. (Amended) The combination according to claim 1, wherein the amphiphilic polymer [of the copolymer] is a polyalkylene oxide.

3. (Amended) The combination according to claim 2, wherein the amphiphilic polymer [of the copolymer] is a polyalkylene glycol.

4. (Amended) The combination according to any one of claims 1 to 3, wherein X or E [in the copolymer] is a charged peptide or peptide derivative.

5. (Amended) The combination according to any one of claims 1 to [4] 3, wherein a ligand for a higher eukaryotic cell is coupled to the copolymer.

6. (Amended) The combination according to any one of claims 1 to [5] 3, wherein the nucleic acid [molecule(s)] molecule is [(are)] condensed with an organic polycation or cationic lipid [molecules] molecule and the complex formed thereby has [one or more] a charged [copolymers] copolymer of the general formula I bound to its surface via ionic interaction.

7. (Amended) The combination according to any one of claims 1 to [6] 3, containing a therapeutically effective nucleic acid molecule.

8. (Amended) The combination according to any one of claims 1 to [7] 3, wherein the carrier consists of a biologically non-resorbable material.

9. (Amended) The combination according to any one of claims 1 to [7] 3, wherein the carrier consists of a biologically resorbable material.

12. (Amended) The combination according to any one of claims 1 to [7] 3, wherein the carrier is a carrier which is obtainable by cross-linkage of a copolymer as defined in claim 1.

13. (Amended) [Use of] A method of transferring a nucleic acid molecule into a cell comprising using a combination according to any one of claims 1 to [12] 3 [for the transfer of a nucleic acid into cells].

14. (Amended) A pharmaceutical composition [containing] comprising a combination according to any one of claims 1 to [12] 3.

15. (Amended) A kit [containing] comprising a carrier and a copolymer or a complex as defined in claim 1.